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EFFECTS OF SOME CALCIUM CHANNEL BLOCKERS ON ISOLATED HUMAN PENILE ERECTILE TISSUES

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ABSTRACT

The effects of the calcium channel blockers (CCBs) verapamil, nifedipine and diltiazem on contractile activation of isolated human penile erectile tissues were investigated. Specimens of the corpus spongiosum (CS) and corpora cavernosa (CC) were obtained from men with a history of normal penile erection undergoing cystourethrectomy because of bladder malignancy. Preparations were mounted in organ baths and isometric tension was recorded. Deprivation of extracellular calcium abolished electrically induced contractions in both CS and CC preparations within 15 min.; norepinephrine (NE)-induced contractions were reduced by 90% (CS) and 83% (CC) after 30 min. All the CCBs reduced electrically induced contractions concentration-dependently, nifedipine being the most potent agent. Contractions induced by exogenous NE were depressed by about 50%, whereas high K^+ (124 mM) induced responses were abolished. It is concluded that contraction in penile erectile tissues is mediated mainly by neuronally released NE stimulating postjunctional alpha-adrenoceptors. The contraction is highly dependent on extracellular calcium and can partly be inhibited by CCBs. It cannot be excluded that some CCBs injected intracavernosally may be useful for diagnosis and even treatment of erectile dysfunction. However, calcium channel blockade may not be as effective as a therapeutic principle as blockade of alpha-adrenoceptors. (*J. Urol., 138: 1267–1272, 1987*)

Future pharmacotherapy of cardiovascular disorders will probably involve an increasing use of calcium channel blockers (CCBs) in patients suffering from angina pectoris and arterial hypertension.[1-3] This means that the drugs will be used in a considerable number of male patients.

The precise mechanisms of penile erection are not yet fully understood, but a relaxation of the smooth muscles of the penile arteries and within the erectile tissues proper is essential.[4-6] Considering their effects on other vascular regions, relaxant effects of CCBs would be expected also in penile erectile tissues. Whether or not such effects would affect penile erectile functions in patients treated systemically with the drugs is not known. A local effect of the CCBs when injected directly into the erectile tissues seems more probable. A recent report suggested that on intracavernosal injection, one such agent, verapamil, may have a clinical potential for the diagnosis and treatment of impotence.[7] More detailed information on the influence of CCBs on penile erectile tissues is obviously desirable, but is, to the best of our knowledge, not available.

The aim of the present study was to obtain information on the extracellular calcium dependence of contractions evoked in the smooth muscles involved in penile erection, and on how these contractions are affected by calcium channel blockade.

MATERIALS AND METHODS

Tissue preparation. Penile tissue was obtained from 20 patients 55 to 77 years old (mean age 68 years), who were undergoing cystourethrectomy because of bladder malignancy, and from one patient (51 years) undergoing penis amputation because of cancer of the penis. Preoperative external radiological treatment (20 Gy) had been given to the patients with bladder malignancy during the week before surgery. Earlier studies suggest that neither age nor external irradiation had any obvious qualitative effect on the responses. All patients had a history of normal penile erection. The specimens were immediately placed in a chilled Krebs solution (for composition, see below) and stored at 4C for up to 24 hours. After removal of connective tissue by sharp dissection specimens of corpora cavernosa (CC) and corpus spongiosum (CS) were dissected into strip preparations, measuring approximately 1 × 2 × 5 mm. A total of 111 CC preparations and 131 CS preparations were investigated.

Recording of mechanical activity. The strip preparations were transferred to 5 ml. organ baths containing Krebs solution (for composition, see below) maintained at 37C by a thermoregulated water circuit, and continuously bubbled with a mixture of CO_2 (5%) and O_2 (95%), resulting in a pH of 7.4. The preparations were suspended between two L-shaped metal prongs by means of silk ligatures. One of the prongs was connected to a Grass Instruments FT03C force-displacement transducer for registration of isometric tension. The other was attached to a movable unit permitting precise adjustment of preload tension. Isometric tension was recorded using a Grass polygraph model 7D.

The preparations were given a one to two hour period of equilibration. During this time, tension was regularly adjusted, and a final tension of 3.5 ± 0.1 mN and 3.8 ± 0.1 mN was achieved for the CC and CS preparations, respectively.

Electrical field stimulation. When subjected to electrical field stimulation the preparations were mounted between two parallel platinum electrodes (four mm. long and three mm. apart) in the organ baths. Transmural stimulation of nerves was performed using a Grass S 48 stimulator delivering single square wave pulses at supramaximum voltage (20 to 30 volts over the electrodes) with a duration of 0.8 ms. The polarity of the electrodes was changed after each pulse by means of a polarity changing unit. Train duration was five s and the stimulation interval 120 to 150 s.

Experimental procedure. Tissue from each patient was studied with all three of the CCBs utilized and no experiment was repeated on tissue from the same patient.

1. Frequency-dependent contractions of the electrically stimulated erectile tissue were recorded. Contractions were evoked by stimulation at frequencies of 1 to 80 Hz. Frequencies of 20

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and 30 Hz were chosen for further investigations of CC and CS preparations, respectively, and were shown to produce reproducible contractions with an amplitude 80 to 90 percent of the maximum response.

To study the effect of calcium removal, the bath medium was changed to a Ca^{2+}-deficient solution, containing 10^{-4} M EGTA. Responses were expressed as a percentage of three reproducible contractions in Ca^{2+}-containing solution.

To study the inhibiting effects of CCBs on the response to electrical field stimulation, nifedipine (10^{-9} M – 10^{-5} M), verapamil (10^{-8} M – 10^{-4} M) and diltiazem (10^{-8} M – 10^{-4} M) were added cumulatively to the preparations. Three consecutive reproducible responses (variation <10%) were required before the next (higher) concentration of a drug was applied. A contact period of at last 15 min. was used for each concentration. To exclude time-dependent changes of the responses to electrical field stimulation, at least one preparation was run in parallel with the experimental preparations receiving no drugs.

2. Norepinephrine (NE) was added cumulatively to the preparations. The concentration of NE was increased only after the response to the previous addition had attained a steady level. The contractile responses were expressed as a percentage of the mean of two consecutive contractions (variation <10%) induced by high K^+ solution (for composition, see below). To study the extracellular Ca^{2+}-dependence and the effect of CCBs, tissues were treated with a Ca^{2+}-deficient solution (containing 10^{-4} EGTA), nifedipine (10^{-6} M and 10^{-5} M), verapamil (10^{-6} M and 10^{-5} M) and diltiazem (10^{-5} M) for 30 min. before the cumulative addition of NE. Two concurrent time-matched controls were exposed only to NE.

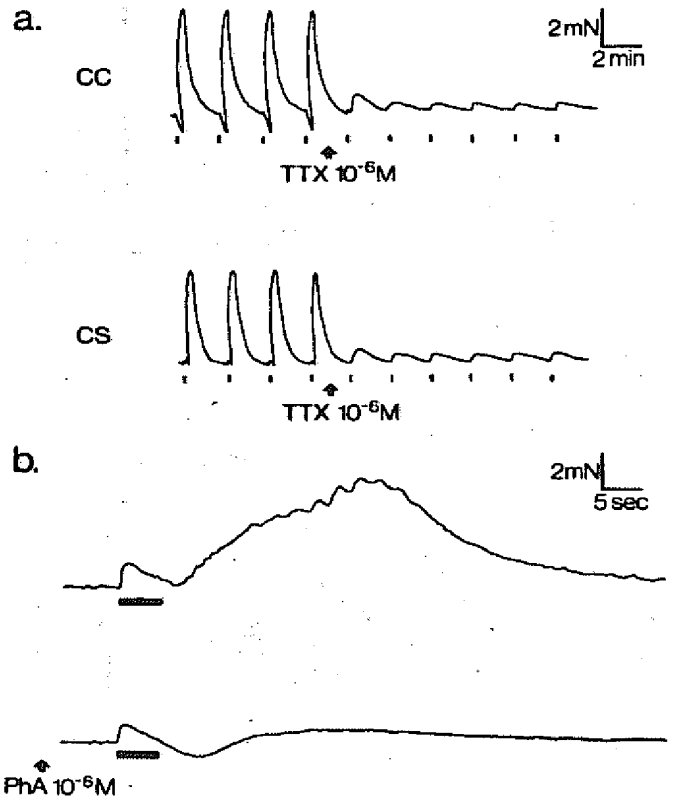

FIG. 1. Tracings of response to electrical field stimulation in isolated preparations from human corpus cavernosum (CC) and corpus spongiosum (CS). Bars indicate duration of stimulation. a) effects of tetrodotoxin (TTX) 10^{-6} M. Stimulation frequencies were 20 Hz in CS and 30 Hz in CC. b) effect of phentolamine (PhA) 10^{-6} M in CC preparation. Stimulation frequency 30 Hz. Note difference in time scale.

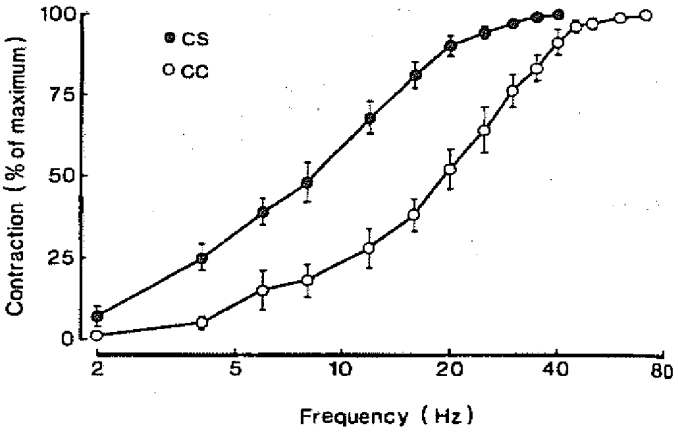

FIG. 2. Frequency-response relations in electrically stimulated, isolated preparations of human corpus spongiosum (CS) and human corpus cavernosum (CC). Each point is expressed as percentage of maximum response obtained in each preparation and represents mean ± standard error of mean of 15 determinations.

3. Contractions induced by a high K^+ solution (124 mM) were recorded. To estimate the contribution by release of endogenous NE, K^+-induced contractions were recorded before and after treatment with phentolamine 10^{-6} M for 30 min.

To study the inhibitory effects of CCBs on K^+-induced contractions in CC and CS preparations, reproducible responses were obtained (variation <10% between two consecutive contractions) at intervals of 40 min. The mean of two of these contractions was used as control and subsequent responses expressed as a percentage of this value. After the control contractions the tissues were exposed for 20 min. to the lowest concentration of nifedipine, verapamil or diltiazem to be tested. The preparations were then exposed in the presence of a CCB to a high K^+-solution. After 40 min. the tissues were again exposed to the high K^+-solution in the presence of the next (higher) concentration of a blocker. A contact period of 20 min. was used for each concentration. The process was repeated until the entire concentration-response curve for a specific CCB was obtained. Only one concentration-response curve was obtained from each strip.

Drugs and solutions. The following drugs were used: phentolamine methane sulphonate (Ciba-Geigy), norepinephrine bitartrate, tetrodotoxin (TTX, Sigma), EGTA (ethyleneglycol bis (β-aminoethylether)-N, N'-tetraacetic acid, Merck), verapamil hydrochloride (Knoll) and diltiazem hydrochloride (Ferrosan). Nifedipine (Bayer) was provided in ampoules, containing 0.1 mg./ml. of nifedipine in 150 mg./ml. of polyethylene glycol and water. The solvent used in the ampoules was shown to have no effect on the mechanical responses in the concentrations used. Care was taken to prevent light-induced degradation of nifedipine by keeping the drug in dark vessels and avoiding direct exposure to light. Stock solutions were prepared and subsequent dilution of the drugs were made with 0.9% NaCl (supplied with one mM ascorbic acid).

The Krebs solution had the following composition (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 11.

High K^+ solution (124 mM) was prepared by replacing sodium for equimolar amounts of potassium in "normal" Krebs solution. Ca^{2+}-free solution refers to Krebs solution from which $CaCl_2$ was omitted, and 0.1 mM EGTA was added.

Statistical analysis. Student's two-tailed test was used for statistical comparison between groups of data. A probability level <0.05 was accepted as significant. When appropriate, results are presented as means ± standard error of the mean. n denotes the number of preparations examined. The log IC_{50}

values (the logarithms of the drug concentrations producing half maximum inhibition) were determined graphically for each curve by linear interpolation.

RESULTS

Response to electrical field stimulation. Electrical field stimulation produced frequency-dependent contractions in both CS and CC preparations, which were abolished by TTX 10^{-6} M (fig. 1). The elicited responses were often biphasic, consisting of an initial rapidly developing component and a second phase starting immediately after stimulation was stopped (fig. 1). Phentolamine 10^{-6} M abolished the responses in the CS and the second phase of the contraction in the CC. The initial contractile response in the CC was unaffected. An initial relaxant component (fig. 1) was found in 15 out of 30 CS preparations and in 18 out of 31 CC preparations. The threshold frequency for contraction was one Hz in the CS and two Hz in the CC. Maximum contraction was obtained at 40 Hz in the CS and at 70 Hz in the CC (fig. 2).

The effect of Ca^{2+} removal on contractions induced by electrical field stimulation after various time periods in a Ca^{2+}-deficient solution (containing 10^{-4} M EGTA) is shown in figure 3. Ca^{2+} deprivation abolished the contractions in both CS and CC preparations after a time period of 15 min. Readmission of Ca^{2+}- containing solution restored the response to control level within five min.

Contraction-response curves for the inhibitory effects of the three CCBs used are shown in figure 4. All blockers concentration-dependently depressed the response to electrical field stimulation, and the order of potency was nifedipine > verapamil > diltiazem. The log IC_{50} values for the respective drugs were in the CS -7.28 ± 0.19, -5.86 ± 0.17, and -5.44 ± 0.20, and in the CC -7.71 ± 0.22, -5.95 ± 0.21, and -4.99 ± 0.17. High concentrations of verapamil and diltiazem (10^{-4} M) were able to abolish the electrically induced contractions, whereas nifedipine in the highest concentration used (10^{-5} M) depressed the response by $78 \pm 4\%$ in the CS and by $74 \pm 4\%$ in the CC.

Response to norepinephrine. Within the concentration range 10^{-9} M to 10^{-4} M NE produced concentration-related increases in tension (fig. 5). The maximum force obtained at 10^{-4} M in CS preparations was 14.5 ± 1.9 mN (n = 15), and in CC preparations 19.5 ± 3.1 mN (n = 16).

In CC and CS preparations, nifedipine, verapamil (at the concentrations 10^{-6} M and 10^{-5} M) and diltiazem (10^{-5} M) depressed the response to NE, but not by more than 50%. No significant differences in effect between the drugs were detected. Pretreatment for 30 min. in a Ca^{2+}-free solution (containing 10^{-4} M EGTA) reduced the NE-induced contraction at 10^{-4} M by 90% in CS preparations and by 83% in CC preparations (fig. 5).

Response to K^+. Exposure to an isotonic high K^+-solution (containing 124 mM K^+) induced reproducible contractions. In CS preparations the response was composed of a rapid increase in tension, which then reached a stable level throughout the K^+ exposure (fig. 6). Phentolamine at the concentration 10^{-6} M (n = 4) did not affect these contractions. CC preparations exposed to high K^+-solution displayed a biphasic contractile response, composed of an initial fast, partly transient contraction and an ensuing, more slowly developing sustained contraction (fig. 6). In CC preparations the first phase of the response to a 124 mM K^+-solution was unaffected by phentolamine 10^{-6} M, while the second phase was substantially reduced (to $74 \pm 4\%$ of control, n = 6). Sample traces showing the effect of phentolamine (10^{-6} M) on the K^+-induced contraction in the

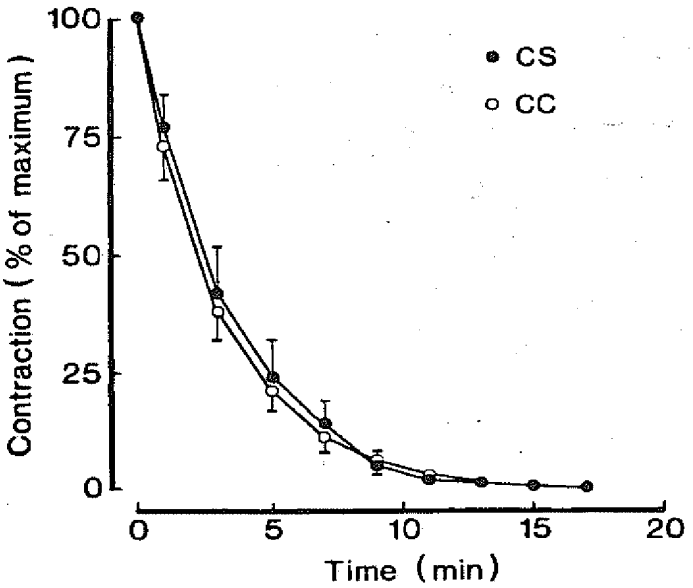

FIG. 3. Relationship between period of incubation in Ca^{2+}-free solution (containing 10^{-4} M EGTA) and amplitude of contraction induced by electrical field stimulation in human corpus spongiosum (CS) and corpus cavernosum (CC). Each point is expressed as percentage of control response in Ca^{2+}-containing solution and represents mean ± standard error of mean of seven determinations.

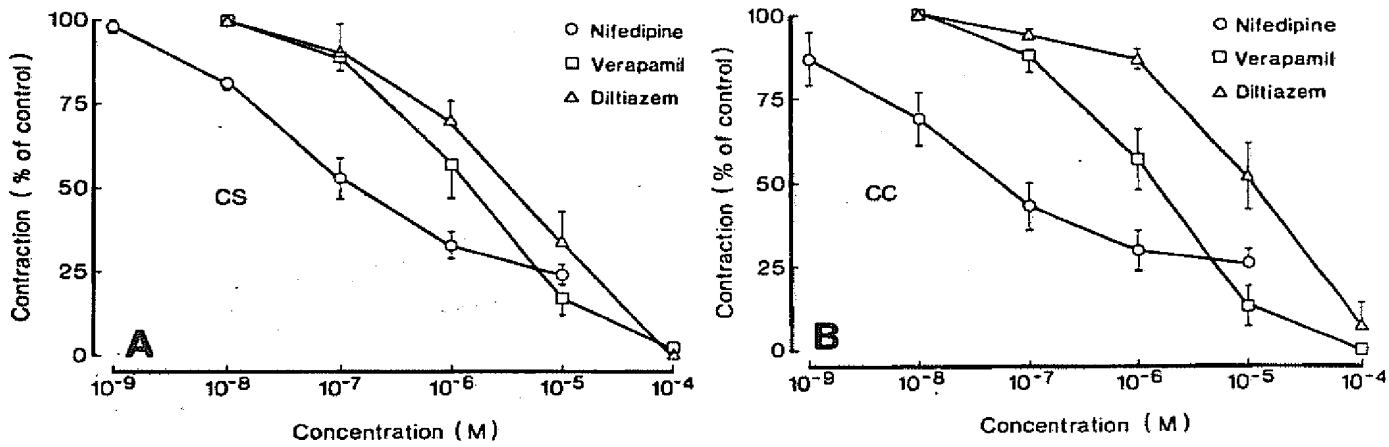

FIG. 4. Inhibiting effects of nifedipine, verapamil and diltiazem on contractions induced by electrical field stimulation in isolated preparations of the human corpus spongiosum (CS) and corpus cavernosum (CC). Each point is expressed as percentage of control response and represents mean ± standard error of mean of six determinations.

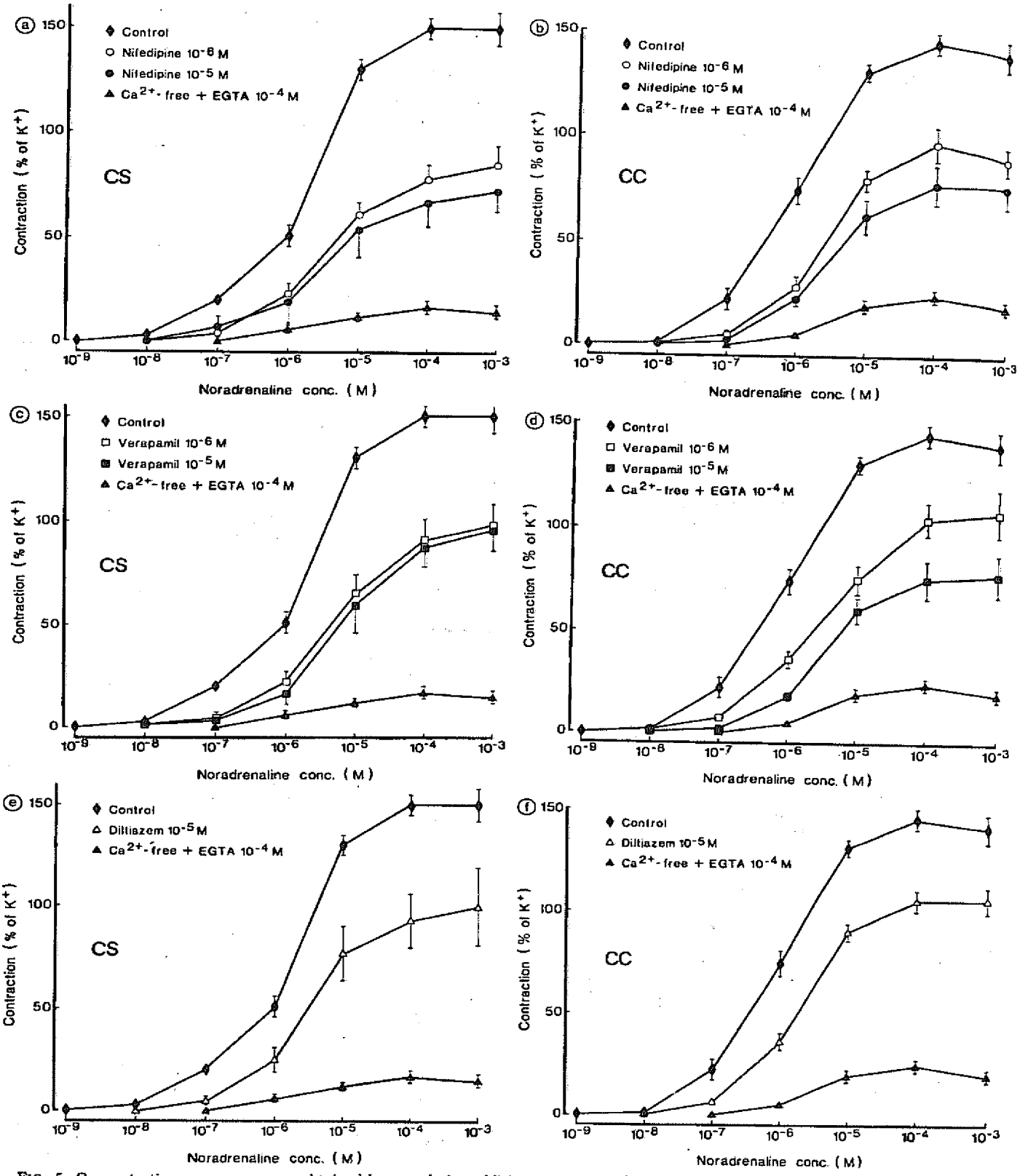

FIG. 5. Concentration-response curves obtained by cumulative addition of norepinephrine to isolated preparations of human corpus spongiosum (CS) and corpus cavernosum (CC) in absence and presence of nifedipine (a, b) verapamil (c, d), diltiazem (e, f) and Ca^{2+}-free solution (10^{-4} M EGTA). Each point is expressed as percentage of maximum control response and represents mean ± standard error of mean of five to seven determinations.

penile erectile tissue is given in figure 6. In the subsequent experiments with high K^+ solution performed on CC preparations, phentolamine 10^{-6} M was present.

Nifedipine, verapamil, and diltiazem reduced the response to 124 mM K^+ in CC and CS preparations concentration-dependently (fig. 7). The log IC_{50} values for the respective drugs were in the CS -7.82 ± 0.15, -6.63 ± 0.13, and -6.20 ± 0.11, and in the CC -7.71 ± 0.29, -6.72 ± 0.18, and -6.01 ± 0.24. Verapamil and diltiazem, but not nifedipine, were significantly more potent inhibiting K^+-induced contractions than contractions induced by electrical field stimulation ($p < 0.05$). Nifedipine 10^{-5} M (the highest concentrations used), caused an almost complete suppression of the K^+-induced contraction, whereas the electrically induced contraction was depressed to a lesser extent (figs. 4 and 7).

DISCUSSION

In agreement with previous results on human erectile tissues,[8] it was shown in the present study that electrical field stimulation elicited a contractile response, which was blocked by tetrodotoxin and alpha-adrenoceptor blockers, although in some preparations of the CC a small initial non-adrenergic component was present. It was also confirmed that exogenous NE produced concentration-dependent contractions in the CC and CS. The findings support the view that the contraction of these tissues, which is necessary for keeping the penis in a flaccid state, is produced mainly by alpha-adrenoceptor stimulation through neuronally released NE.[8,9] However, the differences in frequency-dependence and susceptibility to alpha-adrenoceptor blockade between the CS and the CC suggest diversities in the neurotransmission mediating contraction. The additional neurotransmitter(s) remains, however, unknown.

The present results showed that removal of calcium from the extracellular medium abolished the response to electrical stimulation in both CS and CC preparations. This suggests that contractions evoked by alpha-adrenoceptor stimulation are dependent on extracellular and/or superficially bound membrane calcium in these tissues. The contribution to contraction of intracellularly stored calcium is not known. The electrically induced contractions could be completely blocked by high concentrations (10^{-4} M) of verapamil and diltiazem, but not by the highest concentration (10^{-5} M) of nifedipine used. This difference in effect is probably not attributable to verapamil and diltiazem being more effective CCBs than nifedipine, but to factors other than calcium channel blockade. Thus verapamil, in concentrations exceeding 10^{-5} M, has been shown to possess alpha-adrenoceptor blocking effects[10–12] and diltiazem may have intracellular actions.[12] Nifedipine, which is considered to selectively block calcium entry through voltage sensitive channels, only blocked the electrically induced contraction by about 75% and the NE-induced contraction by about 50%. This is in accordance with the view that NE stimulation of alpha-adrenoceptors may lead to calcium influx also through other (receptor operated) channels, and/or suggests that mechanisms leading to mobilization of intracellularly stored calcium are involved.[13] The contractions by exogenous NE seemed to be more resistant to the CCBs than contractions induced by electrical stimulation. The reason for this can only be speculated upon, but may include differences in the involvement of various activation pathways.

In CC preparations the response to K^+ was depressed by alpha-adrenoceptor blocking drugs. This indicates that part of the response to K^+ in the CC is due to release of NE from adrenergic nerves within the erectile tissue. Alpha-adrenoceptor blockade had no effect on K^+-induced responses in the CS, which may be interpreted to suggest that less NE is released from adrenergic nerves. This in turn, may be due to the occurrence of fewer NE containing nerves in CS tissue. However, if

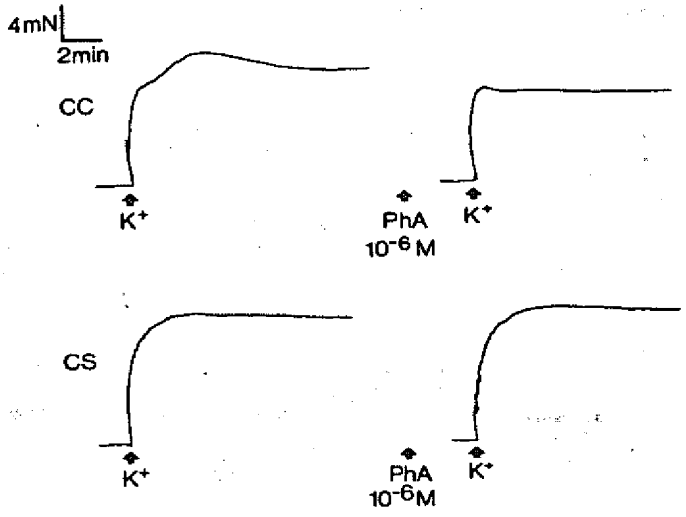

FIG. 6. Effect of phentolamine (PhA) 10^{-6} M on contractile response to K^+ (124 M) in preparations of human corpus cavernosum (CC) and corpus spongiosum (CS). Phentolamine was added 30 min. prior to last exposure to high K^+-solution.

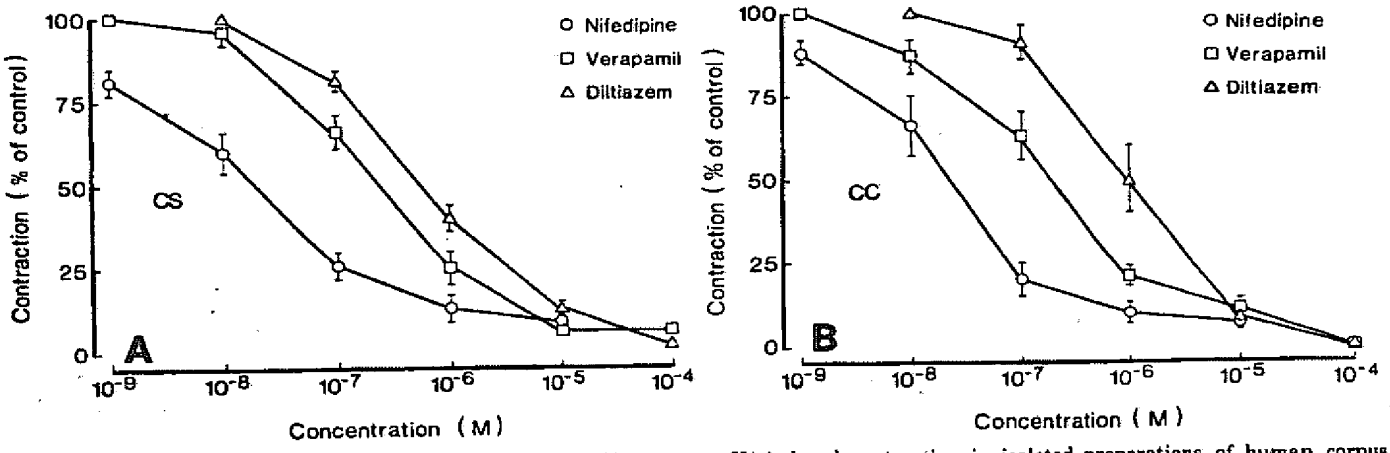

FIG. 7. Inhibitory effects of nifedipine, verapamil and diltiazem on K^+-induced contraction in isolated preparations of human corpus spongiosum (CS) and corpus cavernosum (CC). Each point is expressed as percentage of control response and represents mean ± standard error of mean of six determinations.

there are definite differences in NE content and in density of noradrenergic nerves between CS and CC this has, to the best of our knowledge, not been established. The importance of noradrenergic nerves and NE release for contraction in CS is obvious from the present and previous results. In fact, the frequency response curve in the CS compared with that in CC suggests that electrical stimulation is able to release more NE in the CS than in the CC and/or that CS tissue is the more sensitive to released NE.

Nifedipine, verapamil and diltiazem were all able to abolish the responses induced by K^+ in the mentioned order of potency, which should reflect their blocking potency at voltage-dependent calcium channels.[14] Both verapamil and diltiazem were significantly more potent for inhibition of K^+ than of electrically induced contractions, but were able to abolish both types of contraction at high concentrations. Nifedipine, on the other hand, although the most potent of the drugs, was unable to abolish electrically induced contractions.

Although the neurotransmission in the penile erectile tissues is incompletely understood, the present and previous data suggest that the main part of the contraction is mediated by neuronally released NE stimulating alpha-adrenoceptors. This contraction appears to be highly dependent on extracellular calcium, but can only partly be inhibited by calcium channel blockade.

It may be incorrect to draw conclusions about the in vivo situation from data on isolated tissues, especially as the smooth muscles of the penile arteries and the erectile tissue proper must be regarded as a functional entity. However, if drugs are injected locally, the situation is more similar to that in vitro. The present results suggest that the CCBs' capability of influencing the mechanisms involved in penile erection is questionable at plasma levels obtained at systemic treatment. They may have effects on direct injection into the cavernosal body, but whether nifedipine or diltiazem would be as effective as verapamil cannot be predicted. It should be stressed that the beneficial effects on erection produced by intracorporally injected verapamil,[7] to some extent may be attributable to the alpha-adrenoceptor blocking action of the drug. The present data, suggest that calcium channel blockade can be useful for diagnosis and even treatment of erectile dysfunction. It may not be as effective as alpha-adrenoceptor blockade as a therapeutic principle. However, it cannot be excluded that a combination of the two principles will be clinically useful.

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